

CLAIMS

1. A vaccine composition comprising an isolated protein belonging to the Bcl-2 protein family or an immunogenically active peptide fragment hereof or a nucleic acid encoding
5 said protein or said peptide fragment for use as a medicament.
2. The composition of claim 1, wherein the vaccine composition when administered to a cancer patient, is capable of eliciting an immune response against the cancer disease.
- 10 3. The composition of claim 1, wherein the vaccine composition, when administered to a cancer patient where a Bcl-2 protein family member is expressed, is capable of eliciting an immune response against the cancer disease
4. The vaccine composition according to any of claims 1 to 3, wherein the protein is
15 selected from the group consisting of anti-apoptotic members of the Bcl-2 family.
5. The vaccine composition according to any of claims 1 to 3, wherein the protein is selected from the group consisting of Bcl-2, Bcl-w, Mcl-1, Bfl-1/A1, Bcl-b, Bcl2-L-10 and Bcl-X_L.
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6. The vaccine composition according to any of claims 1 to 3, wherein the protein is selected from the group consisting of Bax, Bok/Mtd, Bad, Bik/Nbk, Bid, Hrk/DP5, Bim, Noxa, Bmf and PUMA/bbc3.
- 25 7. The vaccine composition of claim 5, wherein the protein is Bcl-2.
8. The vaccine composition of claim 5, wherein the protein is Bcl-X_L
9. The vaccine composition according to claim 5, wherein the protein is Mcl-1
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10. An isolated immunogenically active peptide fragment derived from a protein belonging to the Bcl-2 protein family for use as a medicament in the prevention or treatment of a cancer.

11. The peptide fragment according to claim 10, wherein the protein is selected from the group consisting of Bcl-2, Bcl-w, Mcl-1, Bfl-1/A1, Bcl-b, Bcl2-L-10 and Bcl-X_L.
12. The peptide fragment according to claim 10, wherein the protein is selected from the group consisting of Bax, Bok/Mtd, Bad, Bik/Nbk, Bid, Hrk/DP5, Bim, Noxa, Bmf and PUMA/bbc3.
13. The peptide fragment according to claim 11, wherein the protein is Bcl-2
- 10 14. The peptide fragment according to claim 11, wherein the protein is Bcl-X_L
15. The peptide fragment according to claim 11, wherein the protein is Mcl-1
16. The peptide fragment according to any of claims 10 to 15 that is capable of eliciting a cellular immune response in a cancer patient.
- 15 17. The peptide fragment according to any of claims 10 to 16, which is an MHC Class I-restricted peptide having at least one of the following characteristics:
- 20 (i) capable of binding to the Class I HLA molecule to which it is restricted at an affinity as measured by the amount of the peptide that is capable of half maximal recovery of the Class I HLA molecule (C_{50} value) which is at the most 50 μ M as determined by the assembly binding assay as described herein,
- 25 (ii) capable of eliciting INF- γ -producing cells in a PBL population of a cancer patient at a frequency of at least 1 per 10^4 PBLs as determined by an ELISPOT assay, and/or
- (iii) capable of *in situ* detection in a tumor tissue of CTLs that are reactive with the epitope peptide.
- 30 18. The peptide fragment of claim 17 having a C_{50} value, which is at the most 30 μ M.
19. The peptide fragment of claim 17 having a C_{50} value, which is at the most 20 μ M.

20. The peptide fragment of claim 17, which is restricted by a MHC Class I HLA-A molecule.

21. The peptide fragment of claim 20, which is restricted by a MHC Class I HLA species
5 selected from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11 and HLA-A24.

22. The peptide fragment of claim 17, which is restricted by HLA-A2.

23. The peptide fragment according to any of claims 10 to 11 and 16 to 19, which
10 comprises a sequence selected from the group consisting of ALVGACITL (SEQ ID NO:1),
ALSPVPPVV (SEQ ID NO:2), SLALVGACI (SEQ ID NO:3), KTLLSLALV (SEQ ID NO:4),
LLSLALVGA (SEQ ID NO:5), WLSLKTLLSL (SEQ ID NO:6), AAAGPALSPV (SEQ ID
NO:7), PLDFSWLSL (SEQ ID NO:8), FTARGRFATV (SEQ ID NO:9), YLNRHLHTWI
(SEQ ID NO:10), NIALWMTEYL (SEQ ID NO:11).

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24. The peptide fragment according to any of claims 10 to 11 and 16 to 19, wherein the
peptide comprises a sequence selected from the group consisting of TAYQSFEQV (SEQ
ID NO:43), YLNDHLEPWI (SEQ ID NO: 42), RIAAWMATYL (SEQ ID NO:45),
WMATYLNHDL (SEQ ID NO:46), VLVSRIAAWM (SEQ ID NO: 48) and VAFFSFGGAL
20 (SEQ ID NO: 49),.

25. The peptide fragment according to any of claims 10 to 11 and 16 to 19, wherein the
peptide comprises the sequence RIAAWMATY (SEQ ID NO:50).

25 26. The peptide fragment according to any of claims 10 to 11 and 16 to 19, wherein the
peptide comprises a sequence selected from the group consisting of RLLFFAPTR (SEQ
ID NO:55) and RTKRDWLVK (SEQ ID NO:56)

27. The peptide fragment according to any of claims 10 to 11 and 16 to 19, wherein the
30 peptide comprises a sequence selected from the group consisting of PAEEEEDDL (SEQ
ID NO:58) and QSLEIISRY (SEQ ID NO:60).

28 The peptide fragment according to any of claims 10 to 11 and 16 to 19, wherein the
peptide is selected from the group consisting of RLKRDWLVK (SEQ ID NO:62),
35 QSDEIISRY (SEQ ID NO:63) and QSEIISRY (SEQ ID NO:64).

29. The peptide fragment of claim 17, which is restricted by a MHC Class I HLA-B molecule.

5 30. The peptide fragment of claim 29, which is restricted by a MHC Class I HLA-B species selected from the group consisting of HLA-B7, HLA -B35, HLA -B44, HLA-B8, HLA-B15, HLA-B27 and HLA-B51.

31. The peptide fragment according to any of claims 10 to 30 comprising at the most 20
10 amino acid residues.

32. The peptide fragment of claim 31 comprising at the most 15 amino acid residues.

33. The peptide fragment of claim 32, which is a nonapeptide or a decapeptide.
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34. The protein or peptide fragment according to any of claims 10 to 33, which is a native sequence isolated or derived from a mammal species.

35. The protein or peptide fragment according to any of claims 10 to 34 where the protein
20 is a human protein.

36. The protein or peptide fragment according to any of claims 10 to 33, which is derived from a native Bcl-2 protein family member sequence by substituting, deleting or adding at least one amino acid residue.
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37. The peptide fragment according to any of claims 10 to 36 comprising, for each specific HLA allele, any of the amino acid residues as indicated in the following table:

HLA allele	Position 1	Position 2	Position 3	Position 5	Position 6	Position 7	C-terminal
HLA-A1		T,S	D,E			L	Y
HLA-A2		L, M			V		L,V
HLA-A3		L,V,M	F,Y				K, Y, F
HLA-A11		V,I,F,Y	M,L,F,Y, I				K, R

HLA-A23		I,Y				W,I
HLA-A24		Y		I,V	F	I,L,F
HLA-A25		M,A,T	I			W
HLA-A26	E,D	V,T,I,L,F			I,L,V	Y,F
HLA-A28	E,D	V,A,L				A,R
HLA-A29		E				Y,L
HLA-A30		Y,L,F,V				Y
HLA-A31			L,M,F,Y			R
HLA-A32		I,L				W
HLA-A33		Y,I,L,V				R
HLA-A34		V,L				R
HLA-A66	E,D	T,V				R,K
HLA-A68	E,D	T,V				R,K
HLA-A69		V,T,A				V,L
HLA-A74		T				V,L
HLA-B5		A,P	F,Y			I,L
HLA-B7	R,A	P				L,F
HLA-B8			K	K,R		L
HLA-B14		R,K				L,V

HLA-B15 (B62)	Q,L,K,P, H,V,I,M, S,T				F,Y,W
HLA-B17					L,V
HLA-B27	R				Y, K,F,L
HLA-B35	P				I, L, M, Y
HLA-B37	D,E				I,L,M
HLA-B38	H	D,E			F,L
HLA-B39	R,H				L,F
HLA-B40 (B60,61)	E	F,I,V			L,V,A,W, M,T,R
HLA-B42	L,P				Y,L
HLA-B44	E				F,Y,W
HLA-B46	M,I,L,V				Y,F
HLA-B48	Q,K				L
HLA-B51	A,P,G				F,Y,I,V
HLA-B52	Q	F,Y			I,V
HLA-B53	P				W,F,L
HLA-B54	P				
HLA-	P				A,V

B55							
HLA-		P					A,V
B56							
HLA-		A,T,S					F,W,Y
B57							
HLA-		A,T,S					F,W,Y
B58							
HLA-		P					L
B67							
HLA-		R					P
B73							
HLA-		A,L					L
Cw1							
HLA-		A,L					F,Y
Cw2							
HLA-		A,L					L,M
Cw3							
HLA-		Y,P,F					L,M,F,Y
Cw4							
HLA-		Y					L,Y,F,Y
Cw6							
HLA-		Y					L,I,
Cw8							
HLA-		A,L					L,V
Cw16							

38. The peptide fragment according to any of claims 10 to 37 that is capable of eliciting INF- γ -producing cells in a PBL population of a cancer patient at a frequency of at least 10⁵ per 10⁴ PBLs.

39. The peptide fragment according to any of claims 10 to 38, which is capable of eliciting INF- γ -producing cells in a PBL population of a patient having a cancer disease where a protein belonging to the Bcl-2 protein family is expressed.

40. The peptide fragment of claim 39 where the cancer disease is selected from the group consisting of a haematopoietic malignancy including chronic lymphatic leukemia and chronic myeloid leukemia, melanoma, breast cancer, cervix cancer, ovary cancer, lung cancer, colon cancer, pancreas cancer and prostate cancer.

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41. The vaccine composition according to any of claims 1 to 9 comprising the peptide fragment according to any of claims 10 to 40.

42. The vaccine composition of claim 41 that comprises a peptide fragment according to
10 claim 18 in combination with a peptide fragment according to claim 29.

43. The vaccine composition according to any of claims 1 to 9 and 41 to 42 where the vaccine elicits the production in a vaccinated patient of effector T-cells having a cytotoxic effect against the cancer cells.

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44. The vaccine composition according to any of claims 1 to 9 and 41 to 43 further comprising an immunogenic protein or peptide fragment selected from a protein or peptide fragment not belonging to or derived from the Bcl-2 protein family.

20 45. The vaccine composition of claim 44 where the protein or peptide fragment not belonging to or derived from the Bcl-2 protein family is a protein involved in regulation of cell apoptosis or a peptide fragment derived therefrom.

46. The vaccine composition of claim 44 where the immunogenic protein or peptide
25 fragment selected from a protein or peptide fragment not belonging to or derived from the Bcl-2 protein family is survivin or a peptide fragment hereof.

47. The vaccine composition of claim 44 where the immunogenic protein or peptide
fragment selected from a protein or peptide fragment not belonging to or derived from the
30 Bcl-2 protein family is ML-IAP or a peptide fragment hereof.

48. The vaccine composition according to any of claims 1 to 9 and 41 to 47, wherein the composition comprises an adjuvant.

49. The vaccine composition according to claim 48, wherein the adjuvant is selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants and imidazochinilines.

5 50. The vaccine composition according to any of claims 1 to 9 and 41 to 49, wherein the vaccine composition comprises antigen presenting cells comprising the protein or peptide fragment or nucleic acid.

10 51. The vaccine composition according to claim 50, wherein the antigen presenting cell is a dendritic cell.

52. The vaccine composition according to any of claims 1 to 9 and 41 to 51, wherein the composition comprises a liposome.

15 53. The vaccine composition according to claim 1, wherein the nucleic acid encodes the peptide according to any of claims 10 to 40.

54. The vaccine composition according to any of claims 1 and 53, wherein the nucleic acid is comprised within a vector.

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55. The vaccine composition according to claim 54, wherein the vector is selected from the group consisting of viral vectors and bacterial vectors.

25 56. The vaccine composition according to any of claims 54 to 55, wherein the vector furthermore comprises nucleic acids encoding a T-cell stimulatory polypeptide.

57. The vaccine composition according to claim 56, wherein the T-cell stimulatory polypeptide is selected from the group consisting of B7.1, ICAM-1 and LFA-3.

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58. A kit-of-parts comprising the vaccine composition according to any of claims 1 to 9 and 41 to 57, and a further anti-cancer agent.

59. The kit-of-parts according to claim 58, wherein the anti-cancer agent is an antibody

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60. The kit-of-parts according to claim 59, wherein the anti-cancer agent is a cytokine.

61. A composition for *ex vivo* or *in situ* diagnosis of the presence in a cancer patient of T cells in PBL or in tumor tissue that are reactive with a Bcl-2 protein family member, the
5 composition comprising a peptide fragment according to any of claims 10 to 40.

62. A diagnostic kit for *ex vivo* or *in situ* diagnosis of the presence in a cancer patient of T cells in PBL or in tumour tissue that are reactive with a Bcl-2 protein family member, the kit comprising a peptide fragment according to any of claims 10 to 40.

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63. A complex of a peptide fragment according to any of claims 10 to 40 and a Class I HLA molecule or a fragment of such molecule.

64. The complex of claim 63 which is monomeric.

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65. The complex of claim 63 which is multimeric.

66. A method of detecting in a cancer patient the presence of a Bcl-2 protein family member reactive T-cells, the method comprising contacting a tumour tissue or a blood
20 sample with a complex of claim 63 and detecting binding of the complex to the tissue or the blood cells.

67. A molecule that is capable of binding specifically to a peptide fragment according to any of claims 10 to 40.

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68. The molecule of claim 67 which is an antibody or a fragment hereof.

69. The molecule according to claim 67, wherein the molecule is a T-cell receptor.

30 70. A molecule that is capable of blocking the binding of the molecule of claim 67 or 69.

71. A method of treating a cancer disease, the method comprising administering to a patient suffering from the disease an effective amount of the composition according to any of claims 1 to 9 and 41 to 57, the molecule of claim 67 or the molecule of claim 70 or the kit-
35 of-parts according to any of claims 58 to 60.

72. The method of claim 71 wherein the disease to be treated is a cancer disease where a Bcl-2 protein family member is expressed.

5 73. The method of claim 71 wherein the cancer disease is selected from the group consisting of a haematopoietic malignancy including chronic lymphatic leukemia and chronic myeloid leukemia, melanoma, breast cancer, cervix cancer, ovary cancer, lung cancer, colon cancer, pancreas cancer and prostate cancer.

10 74. The method of to claim 71, which is combined with a further cancer treatment.

75. The method of claim 71 wherein the further treatment is selected from the group consisting of chemotherapy, radiotherapy, treatment with immunostimulating substances, gene therapy, treatment with antibodies and treatment using dendritic cells

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76. Use of the peptide fragment according to any of claims 10 to 40 or the vaccine composition according to any of claims 1 to 9 and 41-57 in the manufacture of a medicament for the treatment or prevention of a cancer disease.

20 77. The use of claim 76 wherein the disease to be treated is a cancer disease where a Bcl-2 protein family member is expressed.

78. The use according to any of claims 76-77 wherein the cancer disease is selected from the group consisting of a haematopoietic malignancy including chronic lymphatic leukemia
25 and chronic myeloid leukemia, melanoma, breast cancer, cervix cancer, ovary cancer, lung cancer, colon cancer, pancreas cancer and prostate cancer.

79. The use according to any of claims 76-78, which is combined with a further cancer treatment.

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80. The use of claim 79 wherein the further treatment is selected from the group consisting of chemotherapy, radiotherapy, treatment with immunostimulating substances, gene therapy, treatment with antibodies and treatment using dendritic cells.

35 81. A method of monitoring immunisation, said method comprising the steps of

- i) providing a blood sample from an individual
- ii) providing a protein belonging to the Bcl-2 protein family or a peptide fragment hereof
- iii) determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding the protein or peptide
- iv) thereby determining whether an immune response to said protein or peptide has been raised in said individual.

82. The method according to claim 81, wherein the peptide fragment is a peptide fragment according to any of claims

83. An isolated T-cell comprising a T-cell receptor according to claim 69.